THE RELATION OF STREPTOCOCCI TO BOVINE MASTITIS AND SEPTIC SORE THROAT.

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IN ENGLAND epidemics of sore throat, bearing some relation to the milk supply, have been recognized for many years. The first epidemic was reported as early as 1880 (Rugby). Since then many such epidemics have occurred.

In the United States the first epidemic of sore throat recognized as having a definite relation to the milk supply appeared in Boston in 1911. There is no doubt, I think, that many such epidemics have occurred in the past in this country, as well as in other countries, but on account of the almost universal prevalence of ordinary colds and sore throats their epidemic character and origin were not recognized. In the case of milk epidemics of scarlet fever, diphtheria, and typhoid fever, it may be pointed out that formerly their possible relation to the milk supply was not recognized or was denied, and only recently, when more intensive studies of such epidemics were made, was their true relation to milk supply established.

Since the Boston epidemic of 1911, similar outbreaks have been reported from Chicago, Baltimore, Boston (1912), Concord, N. H., Cortland and Homer, N. Y., Wakefield and Stoneham, Mass., Jacksonville, Ill., and from several other smaller towns especially in the east. The number of persons stricken in these various epidemics has been estimated as follows: Boston 1,400; Chicago 10,000; Baltimore 1,000; Boston (1912) 227; Wakefield and Stoneham 1,000; Cortland and Homer 669, and Jacksonville 348. Probably more than this number were affected since the above are all conservative estimates. This number is sufficient to at least give one some idea of the magnitude and importance of this type of infection.

In all, the onset, the character of the symptoms, and the later complications are strikingly alike and, it may be said, they agree in this respect with similar epidemics in other countries. The relation to the milk supply appears to be unquestionable in all. The interesting fact stands out that there is a certain uniformity in the reports in that the contaminated milk, though used perhaps by a small proportion of the people, still furnished a very high proportion (70, 80, or 90 per cent) of the reported cases. The remainder of the cases probably resulted from personal contact or from some other means.

Streptococci were unquestionably the cause of the disease in all the epidemics, having been found abundantly in the throats or in the secretions of the sick persons in all the cases investigated. This fact is of importance because it establishes definitely the etiology, and since the clinical symp-

toms in all the epidemics are so strikingly uniform, we may consider these infections as a definite clinical entity. They should, I think, take their place and be considered in text-books in medicine along with other infectious diseases, such as scarlet fever, measles, typhoid fever and the like.

As regards the nature of the streptococci, there is a fair degree of uniformity so far as the reports of the various investigators permit one to judge. They are all virulent, usually highly so, for animals. In general, they correspond, with only slight variations, in their morphology, in their cultural characteristics, and in their biological properties. In certain respects there are some slight differences between these streptococci and the ordinary *Streptococcus pyogenes*, and these differences have been sufficient to lead to the use of special terms. It should be stated that it may be questioned whether the differences between them and the *Streptococcus pyogenes* are sufficient to justify such a distinction. They may be simply highly virulent strains of the latter.

One of the properties noted in the streptococci from nearly all the epidemics is that of hemolyzing blood when the colonies are grown on human or rabbit blood agar. While there have been slight variations in the strains studied, they have been strikingly alike in this respect. By hemolysis is meant the formation of a well-defined, wide clear zone about the colonies in 24 hours at incubator temperature. It does not mean a slight halo occurring about the colonies nor does it mean a slight narrow ring of cleared media developing perhaps after 48 hours or more as occurs with certain strains of organisms. This property is of great importance because it is a very ready and practical means of differentiating such organisms from the common Streptococcus lacticus (Bact. guentheri) which is not hemolytic. These latter are practically always present in normal milk, and so far as we know are of no sanitary significance.

It is not to be understood that every hemolytic streptococcus is virulent or dangerous to man. But finding them in any considerable number in milk should make one very suspicious of udder disease, and such milk should at once be excluded from use.

The question of the source of streptococci causing these epidemics of sore throat is an important one. Two possible sources are recognized: the one bovine—the udder or teats of the cow; the other human—some lesion in the throats, hands, etc., of the milker or handler. It is a difficult matter to absolutely prove in a given case whether or not the infection is bovine or human in origin. This is because practically identical hemolytic streptococci occur in the diseased udders of cows and also in the throats and on the hands of the milk handlers. Furthermore, both streptococcal infections of udder in cows and streptococcal infections in the humans are relatively common; consequently, in an investigation of large numbers of cows and of milk handlers, as it is usually necessary to do in studying these epidemics, one is apt to find instances of one or the other and draw conclusions

accordingly. On the other hand, the real source of streptococci may be overlooked on account of some hidden focus of infection in the throat or tonsils of a milker which could not be detected in an ordinary throat examination. Or a cow might be suffering with inflammation of the udder and discharging millions of streptococci in the milk and still, as the writer has shown experimentally, the udder may show no physical signs of the disease and might thereby escape detection on inspection. For these reasons it is readily seen how one might be misled in his conclusions when looking for the ultimate source of streptococci.

I assume that streptococci capable of causing epidemics may at times have their origin in human lesions such as the throat of a milker and through coughing, sneezing, spitting, handling, etc., may pass directly into the milk. This mode of milk contamination we are familiar with in connection with several infectious diseases. I wish here to present experimental evidence showing that it may also be possible for streptococci originating from a person to first infect the teats and udder of the cow, and this source may then continue to furnish virulent streptococci for a long time to the milk.

For some time I have been especially interested in the experimental side of the subject. In our study of the epidemics of sore throat in Chicago in 1911 and in Jacksonville, Ill., in 1914, this question of the susceptibility of the cow to human streptococci arose, as well as the reverse question. In collaboration with Doctor Capps, the writer has shown that hemolytic streptococci of human origin, when introduced by catheter into the healthy udder of a cow, continue to grow and are shed for a considerable period of time, and that their growth is accompanied by certain evidences of mastitis. Experiments made by swabbing human streptococci about the meatus of the healthy teat do not give rise to an ascending infection, but when the teat is injured by scarifying, the streptococci ascend the ducts and cause an infection manifested by marked increase in leucocytes, the presence of large numbers of the hemolytic streptococci but not necessarily by any external physical signs. The streptococci continue to be shed in the milk for a long period of time. In our experiments at the end of four weeks there were still large numbers of streptococci and leucocytes in the milk.

From the above experimental results it would seem that it is possible for streptococci to pass directly into the udder from a human source such as the throat or hands of the milker, to thus infect the cow's udder and after multiplying there pass out into the milk and infect the consumer.

These results have been confirmed and extended recently by Mathers* working with us in Chicago, who has studied especially the behavior and effects of streptococci of human and bovine origin when grown for a long period of time in the udder of the cow. This work is summarized as follows:

Hemolytic streptococci of human origin from sore throat produce mastitis in cows, when injected directly into the milk ducts. This mastitis

*Jour. of Inf. Dis., 1916, Vol. 19, p. 222.

may be severe leading to a caked bag and later to a chronic inflammatory condition which results in an atrophy of the mammary gland. On the other hand, virulent hemolytic streptococci may grow and multiply in the milk ducts of a cow without causing any visible changes in the udder. The milk, however, contains hemolytic streptococci and an increased number of leucocytes. These infections may persist over long periods of time in the form of a chronic mastitis. In three infections at the end of 215, 186, and 146 days, respectively, there was no evidence that these infections were subsiding.

Streptococcus lacticus (nonhemolytic) produces a very acute infection of the udder when cultures are injected directly into the milk ducts. This infection was of short duration (12 days) and left the gland fundamentally unchanged.

A nonpathogenic hemolytic streptococcus of the type commonly found in normal milk may give rise to a transitory inflammation of the udder when injected directly into the milk ducts, producing a mastitis similar in every detail to that produced by nonhemolytic Streptococcus lacticus.

The presence of pathogenic streptococci and an increased number of leucocytes in milk is indicative of a mastitis, and may be the sole indication of mastitis.

The quarters of a cow's udder under experimental conditions are apparently separate as regards infection. One quarter may be infected, while the others remain normal. Examination of the milk from each quarter of the udder is necessary before mastitis can be excluded in a suspected cow.

In three cases of experimental bovine mastitis, all of which were due to hemolytic streptococci with all the characteristics of the human types, no noteworthy changes were observed in the morphology or cultural characteristics of the invading organisms in frequent examinations of the milk throughout the course of the infections. The distinguishing characteristics primarily noted for each organism were still present at the last observation, and there were no modifications which might be considered as indicating change from one type to the other.

The cultural and morphologic characters of Streptococcus lacticus and of the hemolytic streptococcus derived from normal milk did not change any during the course of the udder infections which they induced.

In a recent paper Theobald Smith and J. H. Brown* showed that streptococci, which were the agents of a number of outbreaks of sore throat were "all alike in that the colonies produce immediately around them a clear hemolyzed area on blood agar plates (horse blood)." They state: "Our studies, extending over more than a year and a half, have shown that cultures from throats affected with tonsillitis contained at least two types of streptococci well differentiated on horse blood agar plates. Our attention was largely restricted to one of these types, a streptococcus producing around the colony a clear zone three to four millimeters in diameter. This type corresponds with the hemolytic

*Jour. Med. Res., 1914, 31, p. 455.

strains of the earlier milk-borne epidemics of tonsillitis (Boston, Chicago, and Baltimore). Within each of these groups a close analysis of morphological characters did not bring out differences beyond slight variations in the size of the cocci, but on culture media differences were evident." They divide streptococci on the basis of hemolysis into two types: Type A, in which the colony has a partly discolored and hemolyzed mantle between it and an outer clearer zone; and Type B, in which the colony is surrounded by a clear zone of hemolysis. The former are nearly always nonpathogenic for rabbits; the latter are commonly pathogenic for rabbits except two strains of the B type and possibly one of the A type. Smith and Brown, in looking for a sufficient reason for these explosive epidemics of tonsillitis, in addition to the possible contamination of the milk during milking or later, point to the possibility of the occasional infection of the udder ducts with human streptococci. Such a possibility would be supported by the discovery of hemolytic streptococci of the human type in the milk (Outbreak B) from a cow in the suspected herd; also by the discovery in the mixed milk in the study of another epidemic (Outbreak G) of a strain not distinguishable from human pathogenic strains. The ordinary mastitis or garget streptococci, he infers, are different from streptococci of human tonsillitis and do not cause that infection in man. Only rarely might human types find their way into the ducts through manipulations, and continue to be shed into the milk for some time.

Recently Krumwiede and Valentine* reported a milk epidemic in Rockville Centre, Long Island, in which they found in one cow the wide-zone hemolytic streptococci of the human type. They present evidence which they have interpreted as showing that the epidemic was largely caused by the udder infection of the cow, but that, in conformity with the assumption above discussed, this cow had itself been infected with human streptococci from a milker who was suffering from sore throat.

It should be pointed out that nonhemolytic or feebly hemolytic streptococci commonly cause a distinct and long enduring mastitis in cows. I have isolated a number of such strains in pure culture directly from the inflamed udder of cows. They appear in the gargety milk in long chains and in large numbers. These organisms are harmless for rabbits unless one injects large doses (two or more blood agar slants); then arthritis may develop. My impression from the data in the literature and also from my own experience is that such streptococci are not infrequently the cause of mastitis, but there is no evidence at present to indicate that they are dangerous to man.

Organisms of this type include many of the streptococci in milk usually designated as Streptococcus lacticus. Most of these cause a green discoloration in blood media, but some may be feebly hemolytic, conforming in this regard with Smith and Brown's Type A; others cause no appreciable alteration of the surrounding media.

*Jour. Med. Res., 1915, 33, p. 231.

These organisms likewise are relatively avirulent and so far as is now known, possess no sanitary significance.

In view of these facts I have recently studied further a collection of hemolyzing streptococci from market milk obtained under various conditions including both pasteurized and certified milk. I was especially interested in the question as to whether pathogenic hemolytic streptococci of the human type were found at times in such milk. These streptococci from market milk were also compared with a collection of human hemolytic streptococci as regards various properties especially their pathogenesis for animals.

Especial attention was given to the study of the property of heat resistance on account of its relation to pasteurization.

Only those streptococci were selected whose colonies were surrounded by a distinct clear zone of hemolysis on human blood agar plates (Type B, Theobald Smith).

The feebly hemolytic streptococci (Type A) were often noted in the milk but were not collected and studied since the interest in sore throat epidemics has centered about the cocci with a clear wide zone.

Three hundred and twenty-eight sample specimens of bottled milk were collected from different dairies in the city of Chicago. Excepting 45 samples from one dairy which furnished certified milk all the specimens were pasteurized: and with the exception of two dairies (17 samples) the holding process was used.

The time of the year during which these examinations were made was from October to March.

Blood agar (human) was used in plating and the counts were made after incubation at 37°C. at the end of 48 hours. The colonies of hemolytic streptococci were carefully noted and counted and later their identity was confirmed by further tests.

Eighty-five samples yielded on culture streptococci of the strongly hemolytic variety. The number in different samples varied considerably ranging from a few hundred to several thousand per cc. In the certified milk they were about in the same proportion as in the pasteurized samples. From one dairy in 16 specimens of milk no hemolyzing streptococci were found. In all others some were found.

A study of these 85 strains of hemolyzing streptococci was made as regards their morphology, cultural characteristics and certain other properties. They vary considerably among themselves. They are more resistant to heat than human strains of hemolytic streptococci and possess little or no virulence for rabbits; therefore in all probability not for man. They rapidly acidify and coagulate milk and grow well at 20°C. They may form short or long chains but as seen in milk they often appear in pairs or a chain of a few elements. While they are all definitely hemolytic (Type B, Theobald Smith) the characteristics of the hemolytic zone on plates may vary in certain respects.

These milk strains are different from certain strains of hemolytic streptococci found at times in diseased udders in cows. These latter resemble the strains of hemolytic streptococci from human sources. They are virulent for rabbits.

The question of pasteurization is an interesting one in relation to these infections. In the case of several epidemics in this country the infected milk had been pasteurized by the "flash" method and the evidence in all indicated quite clearly that the milk was contaminated before pasteurization. Nothing further need be said, therefore, concerning the absolute inefficiency of the "flash" method. The harm it may do by giving people a sense of false security is also self evident. In certain epidemics the milk was consumed raw. It would seem that our only safeguard against such epidemics is efficient pasteurization not only of the milk and cream, but also of the material entering into the manufacture of other milk products. It is a point of some importance that it is not uncommon for firms to sell pasteurized milk but to sell cream in the raw state. The latter is of course even more dangerous than milk.

The question as to what constitutes efficient pasteurization for streptococci is one that evidently requires further study. It is commonly stated in the literature that pathogenic streptococci are killed at relatively low temperatures (52–54°C. for 10 minutes, Sternberg). Undoubtedly for many strains this is altogether too low. The recent work of Ayers and Johnson indicated that the thermal death point of typical streptococci varies considerably and one of 22 strains studied by them resisted heating for 30 minutes at 62.8°C. (145°F.) the usual temperature for pasteurization. Furthermore their viability in milk and milk products should be carefully studied since we know that media may exert an important effect on the resistance of organisms to heat.

In my own work in which 98 strains of streptococci were tested none of 24 pathogenic hemolytic streptococci of human origin resisted 60°C. (140°F.) for 30 minutes. Twenty of 74 strains of hemolytic streptococci of milk origin and having practically no virulence resisted 68.3° C. (155°F.) for 30 minutes. I know of no evidence to indicate that strains of streptococci pathogenic to man can resist the usual temperature for pasteurization (145°F. for 30 minutes).